REMARKS/ARGUMENTS

Claims 1-4, 7-11, 14-17 and 27-31 are pending in the application. Claims 3, 5-6, 10, 12-13 and 18-26 have been canceled without prejudice. Claims 1, 8, 16 and 17 have been amended. Reconsideration of the rejection and allowance of claims 1-2, 4, 7-9, 11, 14-17 and 27-31 are respectfully requested.

The Amendment

In order to expedite prosecution of the application and advance the case toward allowance, the claims have been amended. Claims 1, 8 and 17 have been amended to specify that the first filter has a pore size of between about 0.3 and about 1.5 μm. Support for this amendment can be found, for example, on page 7, paragraph 028. Claim 16 has been amended to specify that the preparation is substantially free of contaminating proteins. Support for this amendment can be found, for example, on page 4, paragraph 013. No new matter has been added by this amendment.

Telephonic Interview

The Applicants gratefully acknowledge that the Examiner granted a telephonic interview on March 18, 2005 and has indicated in the Office Action that the unexpected results would be considered further.

35 U.S.C. §103

Claims 1-4, 7-11, 14-17 and 27-31 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Dubensky Jr. *et al.* (USPN 5,789,245, herein "Dubensky") in view of Yu *et al.* (Vaccine (1997) 15(12/13):1396-1404, herein "Yu"), both of record, and further in view of Harley *et al.* (*Clin. Micro. Reviews*, 2001, 14(4):909-932, herein "Harley").

The rejection is respectfully traversed to the extent that the rejection applies to the claims as amended.

Surprising Results

It is known in the art that a low titer of pure virus preparation is a major hurdle to large scale application. The invention improves both, titer and purity of virus product as discussed below. As such, one major advantage of the invention is that the Applicants have developed a method that achieves a very pure virus intermediate through filtering. Notably, the reduction of any residual protein and nucleic acid contamination is important in order to produce a pure virus product that is further applicable to large scale application. For example, the reduction of DNA contamination is a critical step in vaccine production (see FDA publication; *Letter to Sponsors Using Vero Cells as a Cell Substrate for Investigational Vaccine*; 2001; copy enclosed). Particularly, residual DNA (*e.g.*, Vero cell DNA) is of continued concern with respect to viral vaccines according to the Center for Biologics Evaluation and Research (CBER) (see FDA publication, page 1). The Applicants have addressed this concern and have designed a method that achieves a virus preparation which has *less than about 10 pg cellular nucleic acid /µg virus antigen*. The importance of removal of residual DNA from biological products is further emphasized in Smith *et al.* (see *Quantitation of Residual DNA in Biological Products: New Regulatory Concerns and New Methodologies*; Animal Cell Technology: Developments, Processes and Products (1992); Editors R.E. Spier *et al.*; Butterworth-Heinemann; pages 696-698; copy of relevant pages attached). The potential problems associated with such residual DNA include malignant transformation of cells by activated oncogenes, uptake and subsequent expression of viral genomes in cells, and alteration of gene expression by insertion of gene sequences into sensitive control regions of genes (see page 696, second paragraph). The article indicates that although the majority of DNA fragments in residual DNA are too small to harbor complete open reading frames, larger fragments of DNA that are capable of encoding functional proteins are present, and the size distribution of fragments of DNA that are present in the final product will vary with the steps involved in the purification process (see page 697, third paragraph).

Another major advantage of the invention is that the pure virus intermediate of the present invention is *not* substantially reduced during filtration. The Examiner must appreciate that this was unexpected since any filtering process commonly leads to substantial loss of virus

product. The Applicants use a combination of filters that effectively purifies the product without resulting in substantial loss of the intermediate. As such, the Applicants have *surprisingly* found that the enveloped virus passes their filtering system without reduction of virus titer. To that effect, the specification states the following on page 6, paragraph 025:

> It has been **surprisingly** found by the present invention that by filtering the cell culture supernatant derived from cells infected with enveloped viruses (*e.g.*, the Ross River virus), the enveloped virus passes the filter system **without reduction of virus titer**, while cellular contaminants, like proteins and nucleic acid are efficiently removed. The method of the invention provides purification of a high titer virus preparation by filtration, wherein this method is **easily applicable for large-scale purification** and efficiently removes most of the protein derived from the host cells as well as of cellular nucleic acid. The method of the invention therefore provides a process of purifying virus antigen by filtering without remarkable loss of virus titer and virus antigen. [Emphasis added in bold.]

MPEP §2144.08 states that rebuttal evidence may include evidence that the claimed invention yields unexpectedly improved properties or properties not present in the prior art.[1] The Examiner will appreciate that the instant invention achieves *a very pure virus intermediate* through filtering *without* any substantial reduction in virus titer. This is exemplified in Table 1 on page 14, wherein the virus titer ($TCID_{50}$/ml) of the harvest (8.0; 7.6 after separation) was hardly reduced after filtration (7.2). Since the virus titer is shown as $TCID_{50}$/ml, the numbers in the table refer to the following:

8.0 = 1.0×10^8 harvest

7.2 = 1.58×10^7 filtered (= 15.8% $TCID_{50}$ yield)

7.6 = 3.98×10^7 separated

7.2/7.4 = ~2.00×10^7 filtered/Benzonase treated (50% $TCID_{50}$ yield)

Since the Applicants have achieved such a pure virus intermediate (about 97% pure), the final purification method can be selected from any number of methods (*e.g.*, sucrose gradient purification, *etc.*) since residual DNA contamination has already been substantially reduced. In addition, protein contamination in the intermediate product is also substantially reduced through filtering. The Applicants discuss on page 4 of the application (see paragraph

[1] See MPEP §2144.08 (II) (B)

013) that the contaminating proteins and nucleic acids are below the detection limit of the state of the art detection method (*i.e.*, Westernblot analysis and densitometric determination can be used to detect residual proteins while PCR can be used to detect residual DNA).

In comparison, the art uses several steps and/or procedures to achieve a similarly or less pure virus with the added disadvantage that the greater number of steps reduces virus titer and antigen yield. Specifically, the art does not teach a filtering method that achieves a very pure virus intermediate without any substantial reduction in virus titer. As stated on page 6, paragraphs 023 and 024, various methods known in the art are used to remove contaminating products and efficient purification methods often comprise several steps and combinations of methods. Filtration is used in the art to purify biological material, whereby viruses, particularly enveloped viruses, remain in the retenate, and the virus titer in the filtrate is reduced. The Applicants have solved this problem by developing a system that achieves a pure virus preparation without substantial loss of virus titer.

High Purity

The Office Action indicates that the specification defines "purified Ross River Virus antigen" as having greater than about 97% purity as determined by SDS-PAGE and Western blot analysis with anticellular protein specific antibodies and quantification of residual cellular nucleic acid; and that the Applicants point to the viral preparation of greater than about 97% purity following just two filtering steps as proof of surprising results. The Office Action then indicates that the Applicants have not demonstrated that Dubensky's method does not result in an equally pure product. Herein, the Examiner indicates that, although Dubensky describes his product from the filteration steps as "crude", it does not follow that the product was not greater than about 97% pure. The Office Action asserts that it would have been obvious to use a filter pore size of less than 0.65 microns given that the diameter of an alphavirus is known and thus, the determination of the particular filter pore size ranges employed is within the skill of the ordinary worker and a part of the process of normal optimization.

The Office Action is correct in indicating that the purified Ross River Virus antigen is defined as greater than about 97% pure. However, the Examiner may not appreciate

that the Applicants have designed a method that achieves an *intermediate virus product* of about 97% purity. As indicated previously, such a highly purified intermediate product assures a final product of even greater purity (*i.e.*, in addition to no substantial loss in virus titer). This is further explained in the specification on page 7, paragraph 028, which states the following:

> By filtering during virus/virus antigen purification, **substantially all cellular protein contamination is removed.** The cellular **contaminating nucleic acid is also efficiently removed by a factor of at least 35,** and an ***intermediate pure preparation*** having a purity of at least about 97% compared to the starting virus harvest is obtained by this purification step. [Emphasis added in bold.]

Thus, it is clear from the specification that about 97% pure means that the filtering removes nucleic acids by at least a factor of 35 and further removes substantially all cellular proteins. The Examiner will surely appreciate that a 97% pure virus intermediate is a very pure virus preparation considering the current standard in the art which usually achieves virus intermediates of no more than *crude* quality at best (see Dubensky).

> The burden of establishing a *prima facie* case of obviousness falls upon the Examiner. Therefore, the evidence upon which the Examiner relies must clearly indicate that a worker of routine skill in the art would view the claimed invention as being obvious, as meant by 35 U.S.C. §103.[2] [Emphasis added.]

The Office Action speculates that, although Dubensky describes his product from the filteration steps as "crude", it does not follow that the product was not greater than about 97% pure. Yet, the Examiner has provided no evidence why a skilled artisan would ever consider a "crude" virus intermediate as 97% pure. Respectfully, the art understands a "crude" virus intermediate to be a raw or unrefined product that is likely contaminated with substantial amounts of residual protein and DNA. Thus, a "crude" virus intermediate as it appears in Dubensky would never be interpreted as 97% pure by any skilled artisan.

[2] *Ex parte Wolters and Kuypers*, 214 U.S.P.Q. 735 (PTO Bd. App. 1979).

It is also noteworthy, that Dubensky focus on the use of *recombinant viruses as vectors* (see column 1, Technical Field) and *recombinant alphavirus particles* (see column 120) while the Applicants use *inactivated virus as vaccine*. Dubensky teach the preparation of packaged recombinant alphavirus particles in column 120 (Example 10), wherein they state that "the media exiting the bioreactor is collected and passed initially through a 0.8 micron filter, then through a 0.65 micron filter **to clarify** the crude recombinant alphavirus particles" (see column 120, lines 11-14). In comparison, the Applicants use separation (a kind of centrifugation) as the clarification step (see Table 1 on page 14) and filtration to remove DNA and protein. Finally, Dubensky state that DNase is added to digest exogenous DNA (see column 120, lines 16-18) and cross flow filtration is used (diafiltrate is loaded onto a Sephadex-S-500 gel column) to concentrate the virus (see column 120, lines 18-21). However, there is no discussion in Dubensky about the importance of removing residual DNA from the virus preparation nor is any data provided, nor does Dubensky provide any information on the purity of his final product (see column 120, Example 10).

The Office Action then asserts that it would have been obvious to use a filter pore size of less than 0.65 microns given that the diameter of an alphavirus is known and thus, the determination of the particular filter pore size ranges employed is within the skill of the ordinary worker and a part of the process of normal optimization. Herein, the Examiner appears to assume that a small change in a filter range must be interpreted as an obvious change. Yet, there is no such correlation. Even a small change can be a basis for patentability. The Applicants have designed a system with two filter ranges that effectively *purifies* a virus product *without* substantial loss of virus titer. Alpha virus particles are about 400 Å in diameter which amounts to about 0.04 μm. So far, the Examiner has provided no evidence why a second filter of a pore size of between 0.1 μm and 0.5 μm would be obvious in light of Dubensky and/or the alpha virus particle size of 0.04 μm. Dubensky only achieved a crude virus intermediate by using a 0.65 micron filter and he provides no suggestion that a reduced filter size would provide a purer product without substantial loss of virus titer. There is no motivation to combine Dubensky and/or Yu and/or Harley because neither their individual teachings nor their combined teachings would suggest that a filter size in the range of between 0.1 μm and 0.5 μm would lead to a purer

virus product without loss of antigen yield.

The Office further requests clarification with respect to Table 1. The Applicants are requested to explain how the purity went from 97% to just 98% after the benzonase/gradient steps when the amount of VERO cell DNA decreased from 14,000 to [1] 7000 pg/ml. As the Examiner will appreciate, 7000 to 14,000 picograms of DNA are very small amounts (*i.e.*, 7.0×10^{-14} to 14.0×10^{-14} g of DNA). Such a small amount of DNA is not expected to affect the final purity of the virus preparation by more than 1 percent if at all. As explained above, "about 97% pure" means that nucleic acids are removed by at least a factor of 35 and cellular protein contamination is substantially removed as well. In fact, the Examiner must appreciate that the small difference between the 97% pure intermediate virus preparation and 98% final virus preparation only emphasizes that the Applicants have indeed achieved a very pure virus intermediate.

Lastly, the Applicants point out the efficiency of the DNA filtration step for the convenience of the Examiner. Table 1 on page 14 of the specification shows the following information in the last column of the table:

DNA reduction:		**residual DNA**
Harvest	6,300pg/μg	100%
Separator	4,200	33%
Filtration	**175**	**3%**
Benzonase	82	1%
Sucrose gradient	5.5	<< 1%

As the Examiner can see, filtration reduced residual DNA in the virus preparation to as low as 3 percent.

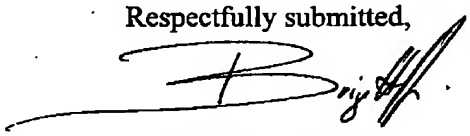
In light of the amendments and arguments presented above, it is respectfully requested that the rejection of claims 1-2, 4, 7-9, 11, 14-17 and 27-31 under 35 U.S.C. §103(a) be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-273-4703.

Respectfully submitted,



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Attachments (2)

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